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PRINCIPAL INVESTIGATOR: Wayne C. Koff, Ph.D.

CONTRACTING ORGANIZATION: United Biomedical, Incorporated
Hauppauge, New York 11788

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Wayne C. Koff, Ph.D.

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Studies undertaken in Year 2 of Grant DAMD 17-95-1-5031 focused on induction of complementary immune responses including neutralizing antibodies, mucosal immunity and cytotoxic T cells likely to be necessary for development of long-term protective immunity against HIV infection and AIDS. Preclinical studies of DNA plasmid immunogens expressing HIV antigens and immunostimulatory cytokines for priming of cellular immune responses followed by subunit peptide immunogens for boosting humoral immunity provided a strategy for maximizing immune effector mechanisms. Similarly, preclinical studies utilizing systemic priming followed by mucosal boosting provided a strategy for addressing differential routes of HIV transmission. Finally, monoclonal antibodies previously identified with potent anti-HIV efficacy, demonstrated their ability to be effective in post-exposure prophylaxis studies, and synthetic immunogens are currently being evaluated to mimic to efficacy of these potent monoclonals.

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Wayne C. Koff 5/27/97

PI - Signature Date

TABLE OF CONTENTS

5. INTRODUCTION

Background (Adapted from Proposal and from 1995-96 Annual Report)

The World Health Organization (WHO) estimates that by the year 2000, 40 million persons worldwide will be infected with human immunodeficiency virus (HIV), the virus which causes AIDS. Despite global investments in HIV prevention and control, HIV infection rates are expanding in several regions of the world. From both a civilian and military perspective, the development of safe and effective HIV vaccines and therapies has become an international priority.

The scientific obstacles to HIV/AIDS prevention and control efforts are many and include: multiple genetic subtypes of HIV circulating worldwide; multiple routes of HIV transmission; efficient transmission of HIV by cell-free and cell-associated virus; lack of complete understanding of which immune responses are required for protective immunity; and the development of virus resistance to licensed antiviral therapies (Myers et al.,1992; Levy, 1992; Koff, 1988; Koff 1994).

United Biomedical, Inc. (UBI®) has developed a comprehensive vaccine and immunotherapy program focused on a multicomponent strategy aimed at eliciting complementary elements of the host immune system i.e. humoral, cellular, and mucosal immune responses which are necessary for effective prophylaxis and treatment of HIV/AIDS. Research supported by Grant # DAMD17-95-1-5031 focuses on two components of our program: 1) Vaccine Development: Immunogen design for induction of mucosal immunity and for induction of long term immune responses through controlled release and 2) Immunotherapeutic Development: Immunogen design for boosting of HIV specific cytotoxic T cell (CTL) responses in HIV infected patients.

Vaccine and Immunotherapeutic Development

During year 1 of Grant # DAMD17-95-1-5031 (May 1, 1995- April 30, 1996), research focused on:

A. Preclinical development of controlled release microparticles for induction of HIV-specific humoral immune responses following a single parenteral immunization.

B. Clinical development of microparticles containing a prototype HIV peptide immunogen, for induction of systemic and mucosal immunity.

C. Development of immunogens capable of stimulating neutralizing antibodies effective against primary isolates of HIV.

D. Designing synthetic lipopeptide immunogens for induction of epitope-specific CTL.

E. Evaluation of peptide mixtures and peptide libraries for induction of CTL.

F. Clinical development of lipopeptides for induction of CTL in HIV seronegative and seropositive individuals.

G. Design of DNA plasmids expressing HIV specific CTL epitopes for induction of CTL.

<u>Summary of major observations from year 1</u> (Adapted from 1995-96 Annual Report)

o Controlled release of synthetic peptide antigens entrapped in microparticles elicited HIV-specific humoral immune respones which could be maintained for approximately one year following a single immunization.

o The results from the first Phase 1 trial of a microparticle entrapped synthetic peptide vaccine (HIV P200M) demonstrated that the microparticles can be safely administered, but were ineffective at stimulating significant levels of either HIV-specific systemic or mucosal immunity.

o A monoclonal antibody was identified with the capability of neutralizing all primary isolates of HIV, generating new targets for HIV vaccine immunogen design.

o Studies with model synthetic peptide antigens demonstrated that when formulated as lipopeptides, epitope specific CTL responses can be induced.

o Preliminary studies demonstrated the feasibility for peptide libraries as immunogens for induction of CTL responses, providing a strategy for addressing epitope variability and escape mutations.

o Data from the first Phase 1 clinical trial of a prototype HIV lipopeptide showed an excellent safety profile and the capacity to stimulate HIV-specific CTL in healthy human volunteers.

o Preliminary studies with DNA plasmids confirmed the feasibility for stimulating CTL with DNA, and provides the opportunity to explore combined regimens of DNA vectors + peptide boosts for optimization of CTL responses.

__Conclusions from Year 1 Studies__ (Adapted from 1995-96 Annual Report).

Studies undertaken in Year 1 of this grant were addressing fundamental immunologic issues central to the development of safe and effective HIV vaccines and immunotherapies, including duration of immunity, mucosal immunity, neutralizing antibodies, and cellular immune responses.

With respect to microparticle studies for induction of mucosal and systemic immunity, significant differences were observed between preclinical studies where microparticle-entrapped immunogens stimulated significant humoral immunity, and the pilot Phase I study which showed transient and low levels of HIV-spepcif immunity. These differences may be related to the route of administration and the capacity of the microparticles to traverse the gastrointestinal system and target Peyers Patches in humans compared with small animal species. In the preclinical studies, microparticles were admininstered via gastrointestinal intubation, whereas the humans swallowed micorparticles dissolved in bicarbonate (Alka-Seltzer) to modulate gastric acid and facilitate gastrointestinal passage. Thus, the effective dose reaching the Peyers patches in human studies may not have been comparable to the preclinical studies.

Development of immunogens capable of stimulating neutralizing antibodies effective against genetically diverse primary isolates of HIV is likely to be an important component for a safe and effective HIV vaccine. The identification of monoclonal antibody 95-29-5, which neutralizes all primary isolates of HIV thus far tested, and has prevented infection of rhesus macaques with the simian equivalent of HIV (SIV), provides a venue for immunogen design. The cellular immunity studies described above demonstrated that synthetic lipopeptides could stimulate epitope specific CTL responses, and that mixtures of lipopeptides and/or peptide libraries are also effective. In addition, the recent preliminary observations that DNA plasmids are efficient inducers of cytotoxic T cell responses, allows for combination studies to be undertaken aimed at optimizing induction of CTL.

Based on these initial observations from year 1 of Grant # DAMD17-95-1-5031, the following goals were proposed for Year 2 of the grant (May 1, 1996-April 30, 1997):

1. Completion of Phase 1 HIV immunotherapy studies using prototype lipopeptide immunogen.

2. Completion of Phase 1 microparticle studies evaluating combinations of oral + parenteral administration of immunogens for induction of systemic and mucosal immunity.

3. Evaluation of methods to optimize CTL reponses utilizing DNA plasmids and synthetic peptide immunogens.

4. Screening of immunogens for induction of neutralizing antibodies against primary HIV isolates, utilizing monoclonal 95-29-5 as the benchmark.

5. Continuation of preclinical studies aimed at improving duration of immunity and stimulation of mucosal immune responses, utilizing polylactide co-glycolide microparticles, and mucosal adjuvants.

6.0 BODY

6.1. Completion of Phase 1 HIV immunotherapy studies using prototype lipopeptide immunogen.

A Phase 1 safety and immunogenicity trial is currently ongoing evaluating the UBI HIV-1 lipopeptide P3C541B in HIV-seropositive human subjects. The subjects for this study comprise HIV-seropositive subjects with CD4 lymphocyte counts above 500. Table 1 provides a summary of the protocol. P3C541B represents a sequence of the HIV-1 gag protein conjugated to Pam3Cys and solubilized in DMSO/Glycerol for parenteral administration.

<u>STUDY DESIGN</u>

The study was designed in two groups. Group 1 consists of of 6 subjects randomized into three groups of two, who received a single subcutaneous injection of either placebo, P3C541B (70ug) or P3C541B (350ug), and were monitored for standard safety parameters but were not evaluated for induction/boosting of CTL responses. Group 2 consists of 12 subjects, randomized in the following manner: 2 placebo; 2 HLA mismatched to receive P3C541B at 350ug, and 8 HLA matched to receive P3C541B at 350ug.

<u>RESULTS</u>

All six subjects of Group 1 were seen on days 0, 14, 28 and 182. The immunogen was well tolerated in all subjects, without any significant adverse reactions. This enabled the study to continue to Group 2. Recruitment for subjects in Group 2 has been slow, perhaps due in part to the recent publicized successes with triple

drug therapy for HIV infection (N. Letvin, Principal Investigator, personal correspondence). Currently, nine subjects have been enrolled in Group 2, and 8/9 have received the first treatment. The immunogen has been well tolerated in all subjects, similar to the observations of Group 1. Preliminary CTL data is available for 5/8 subjects, and is shown in Table 2. No conclusions can be made at the present regarding the capacity of the immunogen to boost CTL responses in HIV seropositive subjects based on either limiting dilution assays (LDA) or bulk CTL, since the study remains blinded and enrollment is not yet complete. Viral load analysis of Group 2 subjects is planned upon completion of enrollment.

6.2. Completion of Phase 1 microparticle studies evaluating combinations of oral + parenteral administration of immunogens for induction of systemic and mucosal immunity.

Preliminary data from a Phase 1 clinical trial of orally administered microparticles containing an entrapped radially branched synthetic peptide vaccine (HIV P200M,) demonstrated that microparticles can be orally administered safely, but were not very effective at stimulating either systemic or mucosal immunity (Adapted from annual report 1995-96). Based on this trial, and studies indicating that p200M was effective at stimulating HIV-specific neutralizing antibodies when administered parenterally (Gorse et. al.), a Phase 1 trial was undertaken to evaluate the safety and immunogenicity of the UBI HIV-1 MN PND radially branched peptide immunogen given by intramuscular injection in combination with microparticles containing this peptide immunogen administered orally in HIV-1 uninfected subjects.

STUDY DESIGN

Table 3 provides an outline of protocol 023, in which 36 subjects were randomized into 2 groups: Group 1 received either 500ug of HIV-MN radially branched V3 PND i.m. or alum placebo in the deltoid, followed by orally administered microparticle boosters at 1, 2, and 8 months. Group 2 received a similar regimen, with the difference being that the i.m. administrations were done in the anterior thigh. The study goals were the following:

o To determine whether oral immunization with microparticles containing HIV-MN V3 PND branched peptide, when preceded by parenteral priming with HIV-MN V3 PND brnached peptide, is safe in healthy HIV-negative adult volunteers.

o To determine if oral immunization when preceded by parenteral priming, provides superior genital mucosal and systemic immune responses relative to an all oral regimen (Clinical trial described in annual report of 1995-96).

o To compare parenteral priming by i.m. injection into the deltoid versus anterior thigh; the latter site drains to inguinal lymph nodes and may induce better mucosal immune responses at genital/rectal mucosal sites.

o To determine whether an accelerated schedule of oral boosts at months 1 and 2 after i.m. priming is sufficient for induction of both genital mucosal and systemic HIV-specific immune responses.

RESULTS

Thirty-six subjects have received the first three immunizations and thirty-four received the fourth (8 month booster). One subject moved and another was lost to follow-up, and thus did not receive the fourth immunization.

Safety Studies: Figures 1 and 2 show the maximum systemic symptoms and maximum pain and tenderness by time after vaccination, and by treatment group. Note that in Figure 2 that pain and tenderness is associated only with the first intramuscular immunization. Table 4 summarizes the safety data, demonstrating that the vaccines were well tolerated, with symptoms among volunteers receiving antigen no worse than among volunteers receiving placebo.

Immunogenicity Studies:

a) ELISA: The immunogenicity of the regimen as measured by peptide ELISA of serum showed only 4/25 (16%) positive responses at four weeks after the last immunization among all antigen recipients. This observation was not significantly different from the 1/16 (6%) positive rate observed in a previous clinical trial when oral administration of immunogen was used for priming.

b) ELISPOTS: ELISPOTS were performed on heparinized peripheral blood specimens using the HIV-1 MN PND branched peptide as antigen. Figure 3 shows the percentage rates of a positive response for IgA, IgG and IgM plotted by weeks post vaccination. Among volunteers receiving a priming injection of HIV-MN PDN in the deltoid, one week after the first oral boost, 33% showed an antigen specific IgA response and 25% showed an antigen specific IgM response. This compares with an 8% response rate for both IgA and IgM from priming injection in the anterior thigh followed by an oral booster.

c) Mucosal binding assays: Table 5 shows the results of assays for specific binding of antibodies present in different mucosal specimens. Using a criterion of 100ng/ml as a threshold for a positive response, and gp120MN as antigen, there have been 25 assays positive for IgA, representing 14 volunteers. Ten subjects

were positive for IgA in the parotid saliva. In contrast, samples from cervical wick, rectal wick, vaginal wash and seminal plasma were negative for IgA at four weeks post the fourth immunization.

6.3. Evaluation of methods to optimize CTL reponses utilizing DNA plasmids and synthetic peptide immunogens.

In year one of this grant, it was demonstrated that synthetic lipopeptides could stimulate epitope specific cytotoxic T cell responses, and that mixtures of lipopeptides were also effective. The recent observations that DNA plasmids are very efficient inducers of CTL in a variety of experimental systems (for review, see Ulmer et. al.), led us to explore the potential for induction of HIV-specific CTL responses with DNA plasmids alone and in combination with cytokines and synthetic peptide boosters.

MATERIALS AND METHODS

Plasmids: The plasmids pCMV 160IIIB encoding the gp160 of HIV-1 IIIB and pcREV encoding HIV rev were described previously (Okuda et. al.). Mouse IL-12 p35 and p40 cDNA individually inserted into pED expression plasmids were provided through a collaboration with Dr. K. Okuda, Yokohama City University School of Medicine.

Mice: Six to ten week old female Balb/c were used for these studies.

DNA Immunizations: Groups of mice were injected once with 160IIIB (2ug DNA), REV (2ug DNA) , and IL-12 (2 to 200ug DNA) expression plasmids into the gastrocnemius muscle in a total volume of 100ul. To facilitate uptake of DNA, muscles were injected with 100ul of 25% sucrose in PBS 30 minutes prior to DNA inoculation.

DTH Studies: Two weeks after immunization, 4ug of HIV-1 IIIB V3 peptide (amino acids RIQRGPGRAFVTIGK) was injected into the footpads, and the amount of footpad swelling was monitored 24 hours later compared with control mice injected with sperm whale myoglobin peptide.

CTL Studies: Three weeks after immunization, spleen cells were isolated and restimulated in vitro with V3 peptide pulsed syngeneic spleen cells. A standard chromium release CTL assay was then performed.

RESULTS

The HIV specific DTH reaction was monitored using the footpad swelling response 2 weeks after immunization. Table 6 shows that a single immunization of the DNA vaccine exhibited significantly

greater swelling compared with a mock vaccine. The DTH response could be augmented when the pCMV 160IIIB/rev was inoculated along with the IL-12 expression plasmid. Similarly, IL-12 augmented the CTL response of the prototype HIV-1 DNA vaccine, as shown in Figure 4. In contrast, data in Table 7 shows that co-inoculation of the HIV DNA vaccine with the IL-12 expression plasmid did not significantly affect the induction of humoral immunity as measured by seum ELISA. The results of the DTH and CTL studies demonstrate that HIV-specific cellular immunity could be enhanced by co-inoculation with an IL-12 expression plasmid.

In an effort to maximize both humoral and cellular immune responses to HIV, preliminary studies were initiated using the pCMV160IIIB/rev plasmid as a priming and V3 peptides as booster immunogens. Table 8 demonstrates that in mice and monkeys (Macaca fuscata, in collaboration with Dr. K. Okuda, Yokohama City University), high levels of HIV-specific antibodies as monitored by V3 peptide ELISA can be maintained for several months by combinations of DNA priming plus peptide booster regimens. These promising preliminary observations suggest that combining DNA plasmids exprssing both HIV specific antigens and immunomodulatory cytokines, plus appropriately designed subunit antigens capable of eliciting cross-reactive neutralizing antibodies, warrants further experimentation with the aim of stimulating the requisite humoral and cellular immunity for protecting against HIV infection and disease.

6.4. Screening of immunogens for induction of neutralizing antibodies against primary HIV isolates, utilizing monoclonal 95-29-5 as the benchmark.

Preliminary data presented in last year's progress report (Adapted from annual report 1995-96) described the development of a monoclonal antibody, termed 95-29-5 (referred below as MAb B4) with the capability of neutralizing primary isolates of HIV-1. This observation led to a series of studies aimed at defining the epitope recognized by B4, and the development of synthetic immunogens capable of eliciting polyclonal antibodies with similar specificity as B4.

MATERIALS AND METHODS

Synthetic peptides. Peptides were synthesized by the Merrifield solid-phase synthesis technique on Applied Biosystems automated peptide synthesizers (Models 430, 431 and 433A) using Fmoc chemistry. After complete assembly of the desired peptide, the resin was treated according to standard procedure using trifluoroacetic acid to cleave the peptide from the resin and deblock the protecting groups on the amino acid side chains. The

cleaved, extracted and washed peptides were purified by HPLC and characterized by mass spectrometry and reverse phase HPLC.

Development of MAb B4. HPB-ALL cell line is a malignant human T cell line derived from a patient with acute lymphoblastic leukemia having the following membrane phenotype revealed by indirect immunofluorescence: CD5+(T1/Leu1+), CD4-expressing (T4/Leu3A+), CD8+(T8/Leu2/C8+), CD3+(T3/Leu4+), CD6+(T6/Leu6+), CD2+(T11/Leu5/D9+), CD25+(Tac+), HLA-A,B,C and $\beta_2$microglobulin+, and HLA-DR-). BALB/c mice were immunized intraperitoneally with 5-10 x 10^6 PBS-washed exponentially growing HPB-ALL cells in complete Freund's adjuvant for the initial immunization followed by weekly to biweekly intraperitoneal boosts with 5-10 x 10^6 PBS-washed exponentially growing cells suspended in PBS without any adjuvant for a total of three months. Splenectomy was performed 3 days after the final intravenous immunization with 5x10^6 PBS-washed HPB-ALL cells and a mononuclear cell suspension was prepared. The mononuclear splenocytes were treated with polyethylene glycol (PEG) for fusion to myeloma cells and somatic cell hybridization. Fusion cells were dispensed into the wells of 96-well microtiter plates, incubated, and wells that contained antibodies specific for rsCD4, as detected by the rsCD4 ELISA described below were selected. The rsCD4-reactive hybridomas were harvested and single-cell cloned by a limiting dilution method in the presence of feeder cells in 96-well, flat-bottomed tissue culture plates. These subcloned hybridomas were subsequently rescreened first for their reactivity with rsCD4 by rsCD4-ELISA and then, rsCD4 reactive clones were further screened for the staining activities of their antibodies with HPB-ALL cells. Only two clones, designated as B4 and M2, having moderate rsCD4 reactivity and staining brightly the HPB-ALL cells were selected for subsequent recloning and maintained as ascites by i.p. injection of 1 x 10^7 cells into nu/nu mice primed with pristane. The binding and neutralization properties of the antibodies secreted by these two clones were further characterized.

rsCD4 ELISA. rsCD4 ELISA were conducted by coating 96-well microtiter plates by overnight incubation at 4°C with rsCD4 at 0.25 µg/mL using 100 µL per well in 10 mM $NaHCO_3$ buffer, pH 9.5. The rsCD4 coated wells were incubated with 250 µL of 3% by weight of gelatin in PBS at 37°C for 1 hr to block non-specific protein binding sites, washed three times with PBS containing 0.05% by volume TWEEN 20 and then dried. Test samples (monoclonal antibodies or a guinea pig anti-rsCD4 serum) were diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20 volume to volume unless indicated otherwise. 100 µL of the diluted sample was added to each of the wells and allowed to react for 1 hr at 37°C. The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS to remove unbound labeled antibodies. 100 µL of horseradish peroxidase labeled goat anti-mouse IgG or goat anti-guinea pig IgG

at a dilution of 1:1000 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37°C for 15 minutes. The wells were washed six times with 0.05% by volume TWEEN 20 in PBS to remove unbound labeled antibody conjugate and reacted with 100 µL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer pH 5.0, for 15 minutes. Reactions were stopped by the addition of 100 µL of 1.0 M H_2SO_4 and the absorbance at 492nm (A_{492}) was measured.

SCID Mice Studies

MAbs. Control antibody used in this study was a murine IgG_{2a}, an antibody of unknown binding specificity, secreted by mouse myeloma cell line RPC5.4 (ATCC No. TIB12). Both B4 and the control IgG_{2a} RPC5.4 were purified from ascitic fluids by Protein-A affinity column chromatography and resuspended in sterile PBS at 2 mg/mL prior to use. All antibodies were given to hu-PBL-SCID mice by intraperitoneal (i.p.) injection.

SCID mouse reconstitution. CB.17 scid/scid mice used in this study were maintained under specific pathogen-free conditions. Nonleaky phenotype mice were reconstituted by i.p. injection of 2 x 10^7 freshly isolated normal human PBL suspended in 0.5 mL of PBS. Two weeks after PBL injection, reconstitution was confirmed by analysis of mouse sera for the presence of human immunoglobulins by ELISA (SangStat, Menlo Park, CA). Only human immunoglobulin-positive mice were used for studies of HIV-1 infection.

Virus stocks. HIV-1 AD6 virus stocks were prepared from the supernatants of infected PBL as described (Ho et al., N Engl J Med, 1989, 321:1621-5) and titrated for infectivity in hu-PBL-SCID mice. They are expressed as the 50% mouse infective dose (MID_{50}) per milliliter.

Virus neutralization assay. HIV-1 neutralizations were performed by a p24 assay as described (Ho et al., J Virol, 1991, 65:489-493). Neutralization was defined as the percent reduction in the amount of p24 antigen released into the culture supernatants from wells treated with antibody compared with control wells not treated with antibody.

Virus challenge of hu-PBL-SCID mice. AD6, known to be resistant to neutralization by most neutralizing antibodies, was selected as the HIV-1 primary isolate in this study. All procedures for infection and maintenance of the hu-PBL-SCID mice were done in a biosafety level 3 animal facility. Infection of hu-PBL-SCID mice was carried out 2 weeks after PBL reconstitution. Mice were injected i.p. with 0.5 mL of diluted cell-free HIV-1 stocks containing 10 MID_{50}. The virus inocula were previously determined by titration in hu-PBL-SCID mice and were shown to infect at least 80% of hu-PBL-SCID mice.

Detection of HIV-1 by coculture. Three weeks after viral

challenge, the mice were sacrificed and cells were recovered from peritoneal lavage and spleens as described (Safrit et al., AIDS 1993, 7:15-21). Then 2×10^5 peritoneal lavage cells or 5×10^6 spleen cells from the mice (with 10-fold serial dilutions) were incubated with 2×10^6 PHA-activated PBL from HIV-1-seronegative human donors in an end-point dilution culture. The cocultures were monitored weekly for the presence of HIV-1 p24 core antigen in the culture supernatant up to 4 weeks. Cultures were considered positive for HIV-1 if a single sample contained >1000 pg/mL or if 2 consecutive samples contained >200 pg/mL p24 antigen. The most highly diluted well containing detectable infected cells was taken as the endpoint, and virus titers were expressed as Tissue Culture Infectious Doses (TCID) per 10^6 cells.

RESULTS

Table 9 summarizes results obtained for the murine monoclonal antibodies developed from three fusion experiments relating to (1) their isotypes; (2) their reactivities with rsCD4 by rsCD4 ELISA; (3) their reactivities with surface CD4 by an indirect immunofluorescence assay registering per cent of cell reactivity, degree of staining (0-3+) and binding patterns; (4) the ability of prior bound HIV gp120 to interfere with binding of the antibodies to host cell antigen complex comprising CD4; (5) the ability of the prior bound antibodies to inhibit binding of HIV gp120 to surface host cell antigen complex comprising CD4, and (6) ability of the antibodies to neutralize HIV-1 primary isolates, e.g., 23135.

Representations of the spatial relationships between HIV gp120 binding sites on cell-membrane bound CD4 and the CD4 epitopes recognized by the various CD4 reactive monoclonal antibodies are specifically addressed by items (3), (4) and (5) of Table 9. Binding patterns to CD4 are shown by indirect immunofluorescence staining of HPB-ALL cells using Fluorescein isothiocyanate (FITC)-labeled anti-mouse IgG, FITC-labeled anti-guinea pig IgG or FITC-labeled gp120. The patterns are shown for binding of anti-CD4 murine monoclonals in the absence of gp120 (3), for the binding of FITC-labeled gp120 in the presence of pre-bound monoclonal antibodies (5), and binding of monoclonal antibodies in the presence of pre-bound gp120 (4). The uniqueness of the neutralization activity of monoclonal antibodies B4 and M2 compared to the neutralization activities of seven other anti-CD4 monoclonal antibodies which have binding sites on CD4 distinct from that of B4 or M2, but in some cases near that of B4, is addressed in items (5) and (6).

Table 10 extends the HIV-1 primary neutralization data of MAb B4 compared with published studies of MAb IgG1 b12 with a number of HIV-1 isolates in H9 and PBMC, and demonstrates the efficacy of B4 in neutralization of HIV-1. Similarly, Table 11 demonstrates the

extends the efficacy of B4 to clades A-F, and shows that mixing of B4 with virus or cell yields the same result with in vitro neutralization studies of MAb B4.
Table 12 demonstrates that B4 is effective in neutralizing HIV-1 when added post-infection, and that this therapeutic effect is dose-dependent. At 2ug/ml, B4 was effective up to 1 hr. post-infection, whereas at 20ug/ml, B4 is effective up to 24 hours post-infection. The mechanism for efficacy of B4 neutralization post-infection has yet to be defined. However, the potent efficacy of MAb B4, coupled with its capacity to neutralize HIV post-infection, led us to consider whether B4 might be interacting with additional host cell receptors. The recent identification of CCR5 as a co-receptor for HIV primary isolates (Deng et. al., Dragic et. al.) led to studies to determine whether MAb B4 was interacting with domains of CCR5 or other chemokine receptors. At various concentrations of B4 between 0.01ug/ml and 10 ug/ml, no interaction was detected between B4 and any of the individual chemokine receptor domain peptides as measured by ELISA. However, when rsCD4 was preincubated with various concentrations of designated chemokine receptor peptides, enhancement of binding of Mab B4 to rsCD4 was observed with chemokine receptor peptides including CCR5 domain 3 (amino acids 168-199). These preliminary observations suggest that the striking efficacy of MAb B4 in neutralization of HIV-1 primary isolates may be associated with its capacity to recognize CD4 in association with other host cell antigens.

Finally, SCID mice protection studies were undertaken with MAb B4 to determine its efficacy in post-exposure prophylaxis.
The post-exposure protection experiments, specifically, were conducted with a B4 concentration of 5 mg/kg for a post-exposure period up to 4 hrs. Mice were challenged intraperitoneally with 10 MID_{50} of HIV-1 primary isolate AD6 prior to administration of any antibody. Mice of control Group 1 (n = 5) were injected intraperitoneally with a 5 mg/kg dose of mouse IgG_{2a} (RPC5.4) immediately following the HIV-1 challenge. Mice of Group 2 (n = 5) were administered 5 mg/kg of MAb B4 immediately following challenge. One, two, and four hrs later, Groups 3, 4 and 5 animals respectively (n = 5 per group) received MAb B4 at a concentration of 5 mg/kg. Each mouse weighed an average of 20 g. Three weeks after viral challenge, the mice were killed and spleen cells and peritoneal lavage were collected for determination of infection by virus culture. As shown in Table 13, HIV-1 was recovered in cultures carried out to 4 weeks for both splenocytes and peritoneal lavage cells that were cocultured with PHA-activated human PBLs from all five mice in group 1 given 5 mg/kg IgG_{2a} (RPC5.4), demonstrating a 100% infectivity rate for this control group. HIV-1 virus was not recovered from any of the twenty mice of Groups 2, 3, 4 and 5 which had been given MAb B4 at 5 mg/kg at 0, 1, 2 or 4 hrs after the viral challenge.

Based on these encouraging observations, preliminary studies have been undertaken to define a synthetic immunogen capable of stimulating polyclonal antibodies which neutralize primary isolates of HIV-1. Currently, several prototype immunogens are being screened in guinea pig immunogenicity studies, and evaluated in primary HIV neutralization screening assays versus HIV-1 clade B isolate 23135. A synthetic immunogen has recently been identified in this preliminary screen which neutralizes HIV-1 clade B 23135, and is currently being further evaluated in neutralization assays versus clades A-F.

6.5. Continuation of preclinical studies aimed at improving duration of immunity and stimulation of mucosal immune responses, utilizing polylactide co-glycolide microparticles, and mucosal adjuvants.

Based on our studies undertaken in Year 1 of this grant (described in Annual Report, 1995-96), significant differences were observed between preclinical studies with microparticle entrapped antigens for induction of mucosal immunity and the transient and low-levels of mucosal immunity oberved in pilot Phase 1 clinical trials. These observed differences might be related to the route of administration and the capacity of the microparticles to traverse the gastrointestinal system and target Peyers patches. In an effort to optimize stragies for induction of mucosal immunity, a collaboration was established between UBI and UC San Diego (Laboratory of Dr. Lynette Corbeil) to evaluate mucosal adjuvants and various routes of immunization using the UBI prototype HIV-1 V3 MN PND vaccine.

<u>MATERIALS AND METHODS</u>

<u>Mice</u>: Balb/c female mice, 6 to 8 weeks of age.

<u>Immunizations</u>: 10ug of HIV-MN peptide vaccine was administered either s.c, orally or vaginally in one of three adjuvants (Alum; Quil A, or cholera toxin). Doses were repeated 3X at monthly intervals.

<u>Immunogenicity Determinations</u>: ELISA and virus neutralization studies were conducted as described above.

<u>RESULTS</u>

Subcutaneous immunization produced significant serum IgG responses, whereas oral or vaginal immunization was ineffective at stimulating serum IgG (Figure 5). In contrast, uterine IgA responses were observed following intravaginal or oral immunization, but not following subcutaneous inoculation (Figure 6). Preliminary studies

with systemic priming followed by intravaginal boosting produced both IgG and IgA in uterine secretions (Figure 7), and these antibodies neutralized HIV-1. These observations suggest that modification of routes of immunization, coupled with utilization of mucosal adjuvants, should be investigated further with the goal of enhancing mucosal immunity against HIV and prevention of the sexual transmission of HIV.

7.0 CONCLUSIONS

A. Summary of major observations from year 2: May '96-April 97.

o Synthetic lipopeptides as immunotherapeutics are well tolerated in HIV-infected subjects; analysis of capability of lipopeptides to boost CTL in HIV-infected subjects is ongoing (Section 6.1).

o Oral immunization of microparticles containing HIV-MN branched peptide, when preceded by parenteral priming of HIV-MN branched peptide, is safe in healthy HIV-negative adult volunteers; Immunogenicity studies of parenteral priming + oral microparticle boosting does not provide superior systemic immune responses compared with oral immunization alone; Antigen specific immune responses as determined by ELISPOT for IgA on heparinizaed peripheral blood specimens and mucosal binding assays for IgA on parotid saliva samples demonstrated the immunogenicity of the parenteral priming + oral micorparticle regimen for generating HIV-specific IgA reponses by synthetic peptide vaccination. (Section 6.2).

o DNA immunization provides a robust strategy for stimulating HIV-specific CTL reponses, which can be augmented by co-administration of plasmids expressing IL-12; Immunization regimens consisting of DNA priming for generating cellular immunity plus peptide boosters for stimulating high levels of humoral immunity warrants further study based on preliminary observations in murine and primate models. (Section 6.3).

o Monoclonal antibody B4 is effective at neutralizing primary isolates of HIV-1 in all clades (A-F) thus far tested; B4 is effective at neutralizing HIV-1 when added pre or post infection, and SCID mice studies demonstrated protective efficacy in a post-infection prophylaxis model; Preliminary findings with a synthetic immunogen derived from binding studies with MAb B4 indicate that it should be feasible to develop immunogens capable of neutralizing

primary isolates of HIV-1. (Section 6.4).

o Preliminary studies aimed at generating mucosal immune responses in the female gential tract by modifying routes of immunization coupled with mucosal adjuvants demonstrated that systemic priming followed by intravaginal boosting is capable of stimulating both HIV-specific IgG and IgA in uterine secretions along with IgG in serum (Section 6.4).

B. Future Directions

Studies being undertaken in this grant are focused on issues central to the development of safe and effective HIV vaccines and immunotherapies, including induction of the requisite cellular, humoral and mucosal immune responses.In the absence of well-defined correlates of protective immunity for HIV infection and AIDS, we have taken the approach to try to maximize the following immune responses:

A. Humoral immune responses capable of neutralizing primary isolates of HIV from divergent internationally circulating clades of HIV.

B. Cellular immune responses capable of conferring long-term memory, and also capable of functionally targeting and killing HIV-infected cells.

C. Mucosal immune responses capable of thwarting infection at local sites of HIV entry, with a specific emphasis on inhibiting sexual transmission of HIV.

Studies in year 2 of this grant have addressed the following common strategies:

o Combinations of systemic priming plus mucosal boosting may be necessary to elicit the targeted immune responses discussed above.

o Combinations of vaccine strategies (e.g. DNA priming + subunit boosting) may be necessary to stimulate high levels of both CTL and neutralizing antibodies.

o Combinations of virus-specific and virus receptor specific antigens may be necessary to elicit high levels of HIV-specific CTL and neutralizing antibodies.

Based on these studies conducted in years 1 and 2 of this grant, the specific aims for Year 3 (1997-1998) will be:

1. Further development of synthetic immunogens capable of neutralizing primary isolates of divergent clades of HIV-1.

2. Combination studies of DNA immunogens + subunit boosts (peptides and lipopeptides) for maximizing CTL and humoral immunity.

3. Combination studies of systemic priming + mucosal boosting utilizing immunogens evaluated in Aim #2 above to generate significantly greater levels of mucosal immunity to supplement cellular and humoral immune responses.

On the basis of data observed from these studies, Year 4 (1998-1999) of the grant would then focus on generation of long term immunity (controlled release studies); primate immunogenicity and protection studies (SIV; SHIV; chimp); and preparations for re-entering clinical trials.

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9.0. APPENDIX

9.1. TABLES

Table 8. DNA priming plus peptide boosting. DNA alone indicates a mixture of pCMV160IIIB and pcREV plasmids. Peptide alone indicates a peptide consisting of the B clade consensus PND and a 13 amino acid residue of the CD4 binding site reoxidized in a cyclized form (Bukawa et. al.). For murine studies, 2ug DNA was injected on days 0 and 14; peptide vaccination was days 0, 14 of 10ug of peptide. DNA+peptide was DNA on day 0 followed by peptide on day 14. Antisera was collected 5 days after the last immunization for ELISA studies. For primate studies, 250ug of pCMV160IIIB and 50ug pcREV was injected at monthly intervals. Peptide alone was immunized monthly for 4 months. DNA+ peptide was DNA 0,1 mo. + peptide months 2,3,4. Antisera was collected at 7 months for ELISA studies.

Table 9: Reactivities of MAbs with rsCD4 and Surface CD4 Receptor

Table 10: Neutralization of HIV-1 by MAb B4.

Table 11: Neutralization of HIV-1 Clades A-F by MAb B4.

Table 12: Post infection neutralization of HIV-1 by MAb B4.

Table 13. Pre and Postexposure Prophylaxis of HIV 1 Infection in Hu-PBL-SCID mice by MAb B4.

9.2 Figures

Figure 1: Maximum systemic symptoms by time after vaccination: Protocol 023.

Figure 2: Maximum pain and tenderness by time after vaccination. Protocol 023.

Figure 3: ELIspot assays: Protocol 023.

Figure 4: CTL of DNA Vaccine +/- IL-12. Three weeks after immunization with pCMV160IIIB/REV and IL-12 expression plasmid, spleen cells were isolated, then restimulated in vitro with HIV-IIIB V3 peptide pulsed syngeneic murine spleen cells for 5 days. The HIV-1 IIIB V3 peptide pulsed p815 cells were used as targets. These data represent the mean of 3-5 experiments.

Figure 5: Serum antibody responses detected by ELISA to HIV-MN V3 branched peptide p200M after immunization of groups of 10 mice with the peptide with alum, quil A or cholera toxin adjuvant by three routes: a) s.c.; b) intravaginal; c) oral. Samples collected every two weeks with immunizations at 0,4,8 weeks.

Figure 6: Antibodies in uterine secretions at termination of the experiment (12 weeks) after three immunizations at monthly

intervals with p200M plus Quil A, alum or cholera toxin (controls were mock immunized normal mice).

Figure 7: Serum, vaginal and uterine IgG and IgA responses after systemic priming (two doses of p200M s.c. one month apart) followed 4 weeks later by intravaginal boosting with 25ug p200M and Quil A.

Table 1

PROTOCOL SUMMARY

Protocol: Phase I safety and immunogenicity trial of United Biomedical, Inc. (UBI®) HIV lipopeptide immunotherapeutic, termed P3C541b, in HIV-seropositive human subjects.

Purpose: To evaluate an HIV lipopeptide immunotherapeutic, at two dose levels, administered subcutaneously (s.c.) in a randomised, single-blinded, placebo-controlled study.

Subjects: The subjects for this study will comprise HIV seropositive volunteers, with CD4 lymphocyte counts above 500/mm^3 on at least a single recent determination within the preceding three months. All prospective subjects will be screened and HLA typed. All acceptable subjects will be allocated to one of two Study Groups and will receive either the placebo, or the immunotherapeutic. Ten subjects possessing one or more HLA types A33, B8, B27, Bw62 will be enrolled in Study Group II (HLA matched), and two further subjects who do not possess one or more of HLA types A33, B8, B27 or Bw62 (HLA mismatched) will be enrolled in study Group II. Study Group I enrollment will be independent of HLA type. Treatment administration for Group II will not begin until Group I participants have reached day 14 without serious adverse events. Enrollment for the study will be considered complete when 18 subjects have been identified.

Schedule:

				Treatment Schedule		
Group	**Dose**	**Number of Subjects**	**Route**	**Day 0**	**Day 28**	**Day 56**
I	Placebo	2	s.c.	X		
I	70μg	2	s.c.	X		
I	350μg	2	s.c.	X		
II	350μg	2 (HLA mismatched)	s.c.	X	X	X
II	350μg	8 (HLA matched)	s.c.	X	X	X
II	Placebo	2	s.c.	X	X	X
Total		**18**				

Estimated Duration: 26 weeks from commencement of treatment.

Principal Investigator and Clinical Site:
Dr. N. Letvin
Division of Viral Pathogenesis
Department of Medicine
Beth Israel Hospital
330 Brookline Avenue
Boston, MA 02215

SUMMARY OF CTL DATA ON GROUP II SUBJECTS IN UBI SEROPOSITIVE PEPTIDE VACCINE TRIAL

SUBJECT	VISIT (date)	LDA (vac-gag targets) (pCTL/10^4PBL)	BULK CTL(peptide targets)(20:1/10:1/5:1/3:1)
CB	screen (1/16)	0.77 (1.17-0.26)	9.6/8.1/6.1/2.6
	screen (2/15)	3.08 (4.34-1.81)	15.7/14.2/9.2/8.3
SMP	screen (1/4)	0.21 (0.36-0.05)	10.1/8.5/5.5/1.9
	14d post-Tx1 (1/18)	10.72 (17.7-0.91)	14.1/8.9/5.7/0.4
	14d post-Tx2 (2/15)	0.42 (1.01-0.01)	7.7/5.7/1.1/0.3
JG	screen (6/24)	7.66 (10.9-3.37)	35.1/43.7/5.4/20.1
	screen (8/26)	33.1 (56.8-22.7)	24.4/12.2/9.1/0.1
	screen (10/3)		11.0/8.7/5.0/1.3
	post-Tx3 (1/2)	0.86 (1.31-0)	2.4/0/2.7/1.2
JWB	screen (6/28)	0.76 (1.3-0.53)	0/0/6.8/2.4
GGA	screen (6/24)	11.88 (25.02-9.27)	38.4/36.6/38.9/24.3
	screen (8/26)	114.9 (99.9-129.9)	51.4/61.8/44.2/29.5
	14dpost-Tx1 (10/13)		56.0/51.0/45.7/36.7
	post-Tx3 (1/2)	3.12 (5.08-1.22)	44.3/43.9/23.3/18.1

Table 2

Table 3

OUTLINE OF PROTOCOL 023

A PHASE I TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF THE UBI® HIV-1 MN PND PEPTIDE IMMUNOGEN, GIVEN BY IM INJECTION, IN COMBINATION WITH THE UBI® MICROPARTICULATE MONOVALENT HIV-1 MN BRANCHED PEPTIDE, GIVEN ORALLY, IN HIV-1 UNINFECTED VOLUNTEERS

Subjects: Subjects for this study will be healthy HIV uninfected (seronegative) adults aged 18-60, with low or intermediate risk for HIV infection; at least 5 of the volunteers in each group (I and II) will be women.

Schema:

		IM PRIME		ORAL BOOST (MONTH)		
Group*	Accrual	Month 0	Injection Site	1	2	8
I	12	500 µg MN V3 peptide in alum	Deltoid	3 mg MN V3 microparticles	3 mg MN V3 microparticles	3 mg MN V3 microparticles
	4	Alum placebo	Deltoid	Microparticulate placebo	Microparticulate placebo	Microparticulate placebo
II	12	500 µg MN V3 peptide in alum	Anterior Thigh	3 mg MN V3 microparticles	3 mg MN V3 microparticles	3 mg MN V3 microparticles
	4	Alum placebo	Anterior Thigh	Microparticulate placebo	Microparticulate placebo	Microparticulate placebo
Total	n = 36**					

* Treatment group omitted to preserve blinding

** Total includes 4 additional volunteers added in 08/95

ACCRUAL AND IMMUNIZATIONS COMPLETED

Product Description: HIV-1 MN PND is a synthetic peptide prototype vaccine based on eight V3-derived homologous peptides attached to a heptalysyl core to form radially branched structures and formulated in alum.

Microparticulate monovalent HIV-1 MN branched peptide vaccine is a branched peptide immunogen consisting of eight V3-derived homologous peptides attached to a heptalysyl core to form radially branched structures, and entrapped in microparticles from polymers, the polylactide co-glycosides and polylactide.

Time Period: First volunteer entered on 05/17/95 and the last on 02/15/96; follow-up of 60 weeks.

Clinical Sites: University of Alabama, University of Rochester and University of Washington

Study Chair: Mark Mulligan, University of Alabama

INCLUSION CRITERIA
Standard inclusion criteria [see Appendix B].

EXCLUSION CRITERIA
Standard inclusion criteria [see Appendix B].

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

MAXIMUM SYMPTOMS SUMMARY
(All Vaccination Visits Combined)

	Placebo (n=8)	Deltoid (n=14)	Thigh (n=14)	Total (n=36)
MALAISE				
None	4	10	10	24
Mild	4	3	3	10
Moderate	0	1	1	2
MYALGIA				
None	7	13	13	33
Mild	1	1	1	3
Moderate	0	0	0	0
HEADACHE				
None	3	9	12	24
Mild	3	4	1	8
Moderate	1	1	1	3
Severe	1	0	0	1
FEVER				
None	7	12	12	31
Mild	0	0	2	2
Moderate	1	2	0	3
NAUSEA				
None	5	8	10	23
Mild	2	6	3	11
Moderate	1	0	1	2
MAXIMUM SYSTEMIC SYMPTOMS				
None	2	7	8	17
Mild	3	5	4	12
Moderate	2	2	2	6
Severe	1	0	0	1

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

MAXIMUM SYMPTOMS SUMMARY
(All Vaccination Visits Combined)

	Placebo (n=8)	Deltoid (n=14)	Thigh (n=14)	Total (n=36)
PAIN				
None	5	6	10	21
Mild	3	7	3	13
Moderate	0	1	1	2
TENDERNESS				
None	7	11	12	30
Mild	1	3	2	6
Moderate	0	0	0	0
MAXIMUM PAIN AND TENDERNESS				
None	4	5	10	19
Mild	4	8	3	15
Moderate	0	1	1	2
ERYTHEMA				
None	7	13	14	34
0-10 cm^2	1	1	0	2
INDURATION				
None	7	14	14	35
0-10 cm^2	1	0	0	1
MAXIMUM TEMPERATURE				
Under 100°F	7	13	13	33
100°F-100.9°F	1	1	0	2
101°F-101.9°F	0	0	1	1
The following are for the three oral immunizations only:				
Abdominal Discomfort				
No	6	11	12	29
Yes	2	3	2	7
Vomiting				
No	8	14	13	35
Yes	0	0	1	1
Altered Bowel Movements				
No	7	10	13	30
Yes	1	4	1	6

Table 5

TABLE 5

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

ELISA DATA FROM THE MUCOSAL IMMUNOLOGY LABORATORY

Protocol	Specimen	Antigen	Treatment	Weeks Post Immunization	IgA +/N	IgA Mean ng/ml in + specimens	IgG +/N	IgG Mean ng/ml in + specimens
					(threshold of 100 ng/ml determines a positive (+) specimen)			
UBI 023	Cervical Aspirate	G-MN120	Alum	2 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	Alum	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	Alum	2 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Deltoid	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Deltoid	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Deltoid	4 weeks post 4th	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Thigh	2 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Thigh	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Thigh	2 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Cervical Aspirate	G-MN120	500 Thigh	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Cervical Wick	G-MN120	Alum	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Cervical Wick	G-MN120	Alum	4 weeks post 4th	0/1	(.)	0/1	(.)
UBI 023	Cervical Wick	G-MN120	500 Thigh	2 weeks post 1st	0/3	(.)	0/3	(.)
UBI 023	Cervical Wick	G-MN120	500 Thigh	2 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Cervical Wick	G-MN120	500 Thigh	4 weeks post 4th	0/3	(.)	0/3	(.)
UBI 023	Intestinal Wash	G-MN120	500 Deltoid	4 weeks post 3rd	0/4	(.)	0/4	(.)
UBI 023	Intestinal Wash	G-MN120	500 Deltoid	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Intestinal Wash	G-MN120	500 Thigh	4 weeks post 3rd	1/4	(283)	0/4	(.)
UBI 023	Intestinal Wash	G-MN120	500 Thigh	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Nasal Wash	G-MN120	Alum	4 weeks post 1st	0/5	(.)	0/5	(.)
UBI 023	Nasal Wash	G-MN120	Alum	4 weeks post 3rd	0/6	(.)	0/6	(.)
UBI 023	Nasal Wash	G-MN120	Alum	4 weeks post 4th	0/4	(.)	0/4	(.)
UBI 023	Nasal Wash	G-MN120	500 Deltoid	4 weeks post 1st	0/9	(.)	0/9	(.)
UBI 023	Nasal Wash	G-MN120	500 Deltoid	4 weeks post 3rd	1/7	(176)	0/7	(.)
UBI 023	Nasal Wash	G-MN120	500 Deltoid	4 weeks post 4th	1/7	(356)	0/7	(.)
UBI 023	Nasal Wash	G-MN120	500 Thigh	4 weeks post 1st	0/8	(.)	0/8	(.)
UBI 023	Nasal Wash	G-MN120	500 Thigh	4 weeks post 3rd	1/10	(200)	0/10	(.)
UBI 023	Nasal Wash	G-MN120	500 Thigh	4 weeks post 4th	1/8	(113)	0/8	(.)
UBI 023	Parotid Saliva	G-MN120	Alum	Pre-immunization	0/7	(.)	0/7	(.)
UBI 023	Parotid Saliva	G-MN120	Alum	4 weeks post 1st	0/7	(.)	0/7	(.)
UBI 023	Parotid Saliva	G-MN120	Alum	4 weeks post 2nd	0/5	(.)	0/5	(.)
UBI 023	Parotid Saliva	G-MN120	Alum	4 weeks post 3rd	0/5	(.)	0/5	(.)
UBI 023	Parotid Saliva	G-MN120	Alum	4 weeks post 4th	1/5	(160)	0/5	(.)
UBI 023	Parotid Saliva	G-MN120	500 Deltoid	Pre-immunization	1/14	(104)	0/14	(.)
UBI 023	Parotid Saliva	G-MN120	500 Deltoid	4 weeks post 1st	0/11	(.)	0/11	(.)
UBI 023	Parotid Saliva	G-MN120	500 Deltoid	4 weeks post 2nd	1/9	(256)	0/9	(.)
UBI 023	Parotid Saliva	G-MN120	500 Deltoid	4 weeks post 3rd	0/10	(.)	0/10	(.)
UBI 023	Parotid Saliva	G-MN120	500 Deltoid	4 weeks post 4th	6/9	(195)	0/9	(.)

Table 5 (Cont'd.)

TABLE 5 - continued

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

ELISA DATA FROM THE MUCOSAL IMMUNOLOGY LABORATORY

Protocol	Specimen	Antigen	Treatment	Weeks Post Immunization	IgA +/N	IgA Mean ng/ml in + specimens	IgG +/N	IgG Mean ng/ml in + specimens
					(threshold of 100 ng/ml determines a positive (+) specimen)			
UBI 023	Parotid Saliva	G-MN120	500 Thigh	Pre-immunization	0/13	(.)	0/13	(.)
UBI 023	Parotid Saliva	G-MN120	500 Thigh	4 weeks post 1st	1/12	(227)	0/12	(.)
UBI 023	Parotid Saliva	G-MN120	500 Thigh	4 weeks post 2nd	1/11	(105)	0/11	(.)
UBI 023	Parotid Saliva	G-MN120	500 Thigh	4 weeks post 3rd	0/11	(.)	0/11	(.)
UBI 023	Parotid Saliva	G-MN120	500 Thigh	4 weeks post 4th	3/8	(178)	0/8	(.)
UBI 023	Preejaculate Swab	G-MN120	Alum	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Preejaculate Swab	G-MN120	Alum	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Preejaculate Swab	G-MN120	Alum	4 weeks post 4th	0/1	(.)	0/1	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Deltoid	4 weeks post 1st	0/4	(.)	0/4	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Deltoid	4 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Deltoid	4 weeks post 4th	0/1	(.)	0/1	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Thigh	4 weeks post 1st	0/3	(.)	0/3	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Thigh	4 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Preejaculate Swab	G-MN120	500 Thigh	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Preejaculate Wick	G-MN120	500 Deltoid	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Preejaculate Wick	G-MN120	500 Thigh	4 weeks post 4th	0/1	(.)	0/1	(.)
UBI 023	Rectal Wick	G-MN120	Alum	4 weeks post 1st	0/6	(.)	0/6	(.)
UBI 023	Rectal Wick	G-MN120	Alum	4 weeks post 3rd	0/6	(.)	0/6	(.)
UBI 023	Rectal Wick	G-MN120	Alum	4 weeks post 4th	0/5	(.)	0/5	(.)
UBI 023	Rectal Wick	G-MN120	500 Deltoid	4 weeks post 1st	0/13	(.)	0/13	(.)
UBI 023	Rectal Wick	G-MN120	500 Deltoid	4 weeks post 3rd	0/9	(.)	0/9	(.)
UBI 023	Rectal Wick	G-MN120	500 Deltoid	4 weeks post 4th	1/4	(137)	0/4	(.)
UBI 023	Rectal Wick	G-MN120	500 Thigh	2 weeks post 1st	0/2	(.)	0/2	(.)
UBI 023	Rectal Wick	G-MN120	500 Thigh	4 weeks post 1st	1/8	(191)	0/8	(.)
UBI 023	Rectal Wick	G-MN120	500 Thigh	2 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Rectal Wick	G-MN120	500 Thigh	4 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Rectal Wick	G-MN120	500 Thigh	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Semen	G-MN120	Alum	4 weeks post 1st	0/4	(.)	0/4	(.)
UBI 023	Semen	G-MN120	Alum	4 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Semen	G-MN120	Alum	4 weeks post 4th	1/1	(103)	0/1	(.)

TABLE 5 - continued

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

ELISA DATA FROM THE MUCOSAL IMMUNOLOGY LABORATORY

Protocol	Specimen	Antigen	Treatment	Weeks Post Immunization	IgA +/N	IgA Mean ng/ml in + specimens	IgG +/N	IgG Mean ng/ml in + specimens
					(threshold of 100 ng/ml determines a positive (+) specimen)			
UBI 023	Semen	G-MN120	500 Deltoid	4 weeks post 1st	0/4	(.)	0/4	(.)
UBI 023	Semen	G-MN120	500 Deltoid	4 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Semen	G-MN120	500 Deltoid	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Semen	G-MN120	500 Thigh	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Semen	G-MN120	500 Thigh	4 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Seminal Plasma	C-GP120	500 Deltoid	4 weeks post 1st	0/2	(.)	0/2	(.)
UBI 023	Seminal Plasma	G-MN120	Alum	4 weeks post 1st	0/3	(.)	0/3	(.)
UBI 023	Seminal Plasma	G-MN120	Alum	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Seminal Plasma	G-MN120	Alum	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Seminal Plasma	G-MN120	500 Deltoid	4 weeks post 1st	1/5	(318)	0/5	(.)
UBI 023	Seminal Plasma	G-MN120	500 Deltoid	4 weeks post 3rd	0/4	(.)	0/4	(.)
UBI 023	Seminal Plasma	G-MN120	500 Deltoid	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Seminal Plasma	G-MN120	500 Thigh	4 weeks post 1st	0/3	(.)	0/3	(.)
UBI 023	Seminal Plasma	G-MN120	500 Thigh	1 week post 2nd	0/1	(.)	0/1	(.)
UBI 023	Seminal Plasma	G-MN120	500 Thigh	4 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Seminal Plasma	G-MN120	500 Thigh	4 weeks post 4th	0/3	(.)	0/3	(.)
UBI 023	Seminal Plasma	M-MN160	500 Deltoid	4 weeks post 1st	0/2	(.)	0/2	(.)
UBI 023	Seminal Plasma	Mp24	500 Deltoid	4 weeks post 1st	1/2	(125)	0/2	(.)
UBI 023	Seminal Plasma	P-NB160	500 Deltoid	4 weeks post 1st	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	Alum	2 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Vaginal Wash	G-MN120	Alum	4 weeks post 1st	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	Alum	2 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	Alum	4 weeks post 3rd	0/1	(.)	0/1	(.)
UBI 023	Vaginal Wash	G-MN120	Alum	4 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	500 Deltoid	2 weeks post 1st	0/4	(.)	0/4	(.)
UBI 023	Vaginal Wash	G-MN120	500 Deltoid	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Vaginal Wash	G-MN120	500 Deltoid	2 weeks post 3rd	0/3	(.)	0/3	(.)
UBI 023	Vaginal Wash	G-MN120	500 Deltoid	4 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	2 weeks post 1st	0/7	(.)	0/7	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	4 weeks post 1st	0/1	(.)	0/1	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	2 weeks post 3rd	0/6	(.)	0/6	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	4 weeks post 3rd	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	2 weeks post 4th	0/2	(.)	0/2	(.)
UBI 023	Vaginal Wash	G-MN120	500 Thigh	4 weeks post 4th	0/4	(.)	0/4	(.)

Table 5 (Cont'd.)

Table 6: Footpad swelling response of mice coinoculated with DNA vaccine and IL-12 expression plasmid[a]

Immunogens	Swelling Response ($\times 10^{-2}$ mm): IIIB V3 Peptide	Swelling Response ($\times 10^{-2}$ mm): Myoglobin peptide
pCMV160IIIB/REV		
Alone	11.2 ± 3.4*	1.7 ± 1.2
With pEDIL-12		
2 μg	12.7 ± 1.9*	ND
20 μg	18.5 ± 3.4*,**	2.2 ± 0.9
200 μg	13.1 ± 3.4*	ND
With pEDempty	9.3 ± 1.6*	ND
pCMVempty	1.8 ± 1.2	1.6 ± 1.3
None	1.3 ± 0.8	1.7 ± 0.9

a BALB/c mice were i.m. inoculated once with 2 μg of pCMV160IIIB/REV alone or with various doses of IL-12 expression plasmids. Four weeks after vaccination, the footpad swelling assay was carried out. The response of each group represents the mean ±SE obtained in five to seven mice.

* and ** indicate mean values significantly different from those of mice injected with pCMVempty)$p<0.001$) and from those of mice injected with pCMV160IIIB/REV ($p<0.05$), respectively.

Table 7: HIV-1-specific Ab titers of mice coinoculated with the DNA vaccine and IL-12 expression plasmid[a]

Immunogens	IgG	IgG1	IgG2a
pCMV160IIIB/REV			
Alone	8.1 ± 0.4	7.9 ± 0.6	3.9 ± 0.6
With pCAGGSIL-12	8.4 ± 0.5	7.7 ± 0.8	4.3 ± 1.7
pCMVempty	ND[b]	ND	ND

a BALB/c mice were i.m. inoculated once with 2 μg of pCMV160IIIB/REV alone or combined with 20 μg of pCAGGSIL-12. Four weeks after immunization, anti-HIV-1 IgG, Ig1 and Ig2a titers in sera were assayed by ELISA. The titer of each group represents the mean ±SE (reciprocal log, titer) obtained in five to seven mice in three independent experiments.

b Not detected

Table 8: DNA Vaccine Priming Plus Peptide Boosting

	ELISA Titers	
	Mice	**Monkeys**
DNA	128	1,024
Peptide Alone	1,024	16,384
DNA + Peptide	2,048	65,536

Table 7

Reactivities of MAbs with rsCD4 and Surface CD4 Receptor Complex and Inhibition of HIV gp120 Binding to CD4 Cells by MAbs

Clone	(1) Murine Ig isotype	Immunogen	(2) $A_{492\ nm}$ Reactivity to rsCD4	(3) HPB-ALL surface CD4 binding pattern	(4) MAb binding reactivity to HPB-ALL after prior binding of gp120 to the cells Inhibition Yes or No	(5) gp120-FITC binding to HPB-ALL after prior binding with MAb Inhibition Yes or No	(6) 50% end point for neutralization of HIV-1 primary isolate (23135)
B4	γ2a,κ	HPB-ALL (PBS)	1.424	>90%; 3+(A)	>90%; 2+(A); Yes, partial	0; Yes	0.21 μg/mL
M2	γ1,κ	HPB-ALL (PBS)	0.871	>90%; 3+(A)	>90%; 2+(A); Yes, partial	0; Yes	0.38 μg/mL
E6	γ1,κ	rsCD4 (CFA, ICFA)	2.007	>90%; 2+(B)	0; Yes	0; Yes	59 μg/mL
H5	γ1,κ	rsCD4 (CFA, ICFA)	1.984	>90%; 1+(B)	>90%; 1+(B); No	>90%; 1+(C); No	45.5 μg/mL
E31	γ1,κ	rsCD4 (CFA, ICFA)	0.936	>90%; 1+(B)	>90%; 1+(B); No	>90%; 1+(C); No	>100 μg/mL
J33	γ1,κ	rsCD4 (CFA, ICFA)	2.059	>90%; 1+(B)	>90%; 1+(B); No	>90%; 1+(C); No	>100 μg/mL
D5	γ1,κ	HPB-ALL (CFA,ICFA) + gp120 rsCD4 complex	1.930	>90%; 1+(B)	0	>90%; 1+(C); No	>10 μg/mL
E2	γ1,κ	HPB-ALL (CFA,ICFA) + gp120 rsCD4 complex	2.020	10%; 1+(B)	0	>90%; 1+(C); No	>10 μg/mL
I26	γ1,κ	HPB-ALL (CFA,ICFA) + gp120 rsCD4 complex	0.793	0	0	>90%; 1+(C); No	>10 μg/mL
PBS	N/A	N/A	N/A	N/A	N/A	>90%; 1+(C); No	N/A
gpα rsCD4	N/A	rsCD4 (CFA,ICFA)	Log_{10} Titer=>5	>90%; 1+(B)	>90%; 1+(B); No	ND	<1:10 dilution

Binding patterns: A: caps and patches; B: patches; C: clusters (Figure 3).

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Table 10: Neutralization of HIV-1 by MAb B4

Virus	Virus Host	μg/mL @ 90% neutralization	
		IgG1 b12	B4
IIIB	H9	0.04	>100
MN	H9	0.91	67
VL135	PBMC	50	0.84
VL114	PBMC	22	0.56
VL172	PBMC	>200	0.39
VL069	PBMC	>50	>100
VL750	PBMC	>200	2

Table 11: Neutralization of HIV-1 Clades A-F by MAb B4

HIV-1		μg/mL of MAb B4 @ 90% neutralization	
Subtype	Strain	MAb + virus	MAb + cell
A	UG/92/029	3.9	3.6
B	DH12	1.9	3.7
B	VL135	0.9	0.8
B	BR/92/014	1.2	ND
C	ZIM748	3	0.35
D	UG266	18	35
E	TH32036	1.8	0.81
F	BR/93/020	15	18

plaque reduction assay in MT-2 cells

Table 12: Post infection neutralization of HIV-1 by MAb B4

B4 (μg/mL)	B4 added (hours post infection)	pfu (% of control)		
		1:300 virus	1:150 virus	1:75 virus
2	0	0	0	0
2	1	0	0	0
2	2	2	2	4
2	6	29	16	17
2	24	59	64	87
20	0	0	0	0
20	1	0	0	0
20	2	0	0	0
20	6	0	0	0
20	24	0	0	0

(HIV-1 (VL 135) in MT-2 plaque reduction assay)

Experiment 3
Pre- and Postexposure Prophylaxis of HIV-1_{AD6} Infection
in Hu-PBL-SCID mice by MAb B4

	Experimental Groups	HIV-1 recovery from hu-PBL-SCID mice: WEEK 4 Co-culture			
		Peritoneal Lavage	Spleen	Culture end-point	TCID/10^6 cells
1	Murine IgG_{2a} (RPC 5.4) (5 mg/kg dose), 0 hr after challenge				
	4630	+	+	$5x10^5$	2
	4634	+	+	$5x10^1$	20,000
	4647	+	+	$5x10^1$	20,000
	4652	+	+	$5x10^5$	2
	4664	+	+	$5x10^1$	20,000
2	MAb B4 (5 mg/kg dose), 0 hr after challenge				
	4636	-	-	-	<0.2
	4639	-	-	-	<0.2
	4640	-	-	-	<0.2
	4660	-	-	-	<0.2
	4666	-	-	-	<0.2
3	MAb B4 (5 mg/kg dose), 1 hr after challenge				
	4643	-	-	-	<0.2
	4644	-	-	-	<0.2
	4645	-	-	-	<0.2
	4646	-	-	-	<0.2
	4648	-	-	-	<0.2
4	MAb B4 (5 mg/kg dose), 2 hrs after challenge				
	4642	-	-	-	<0.2
	4649	-	-	-	<0.2
	4650	-	-	-	<0.2
	4653	-	-	-	<0.2
	4656	-	-	-	<0.2
5	MAb B4 (5 mg/kg dose), 4 hrs after challenge				
	4651	-	-	-	<0.2
	4654	-	-	-	<0.2
	4657	-	-	-	<0.2
	4658	-	-	-	<0.2
	4659	-	-	-	<0.2

Figure 1

FIGURE 1

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]
MAXIMUM SYSTEMIC SYMPTOMS
BY TIME AFTER VACCINATION

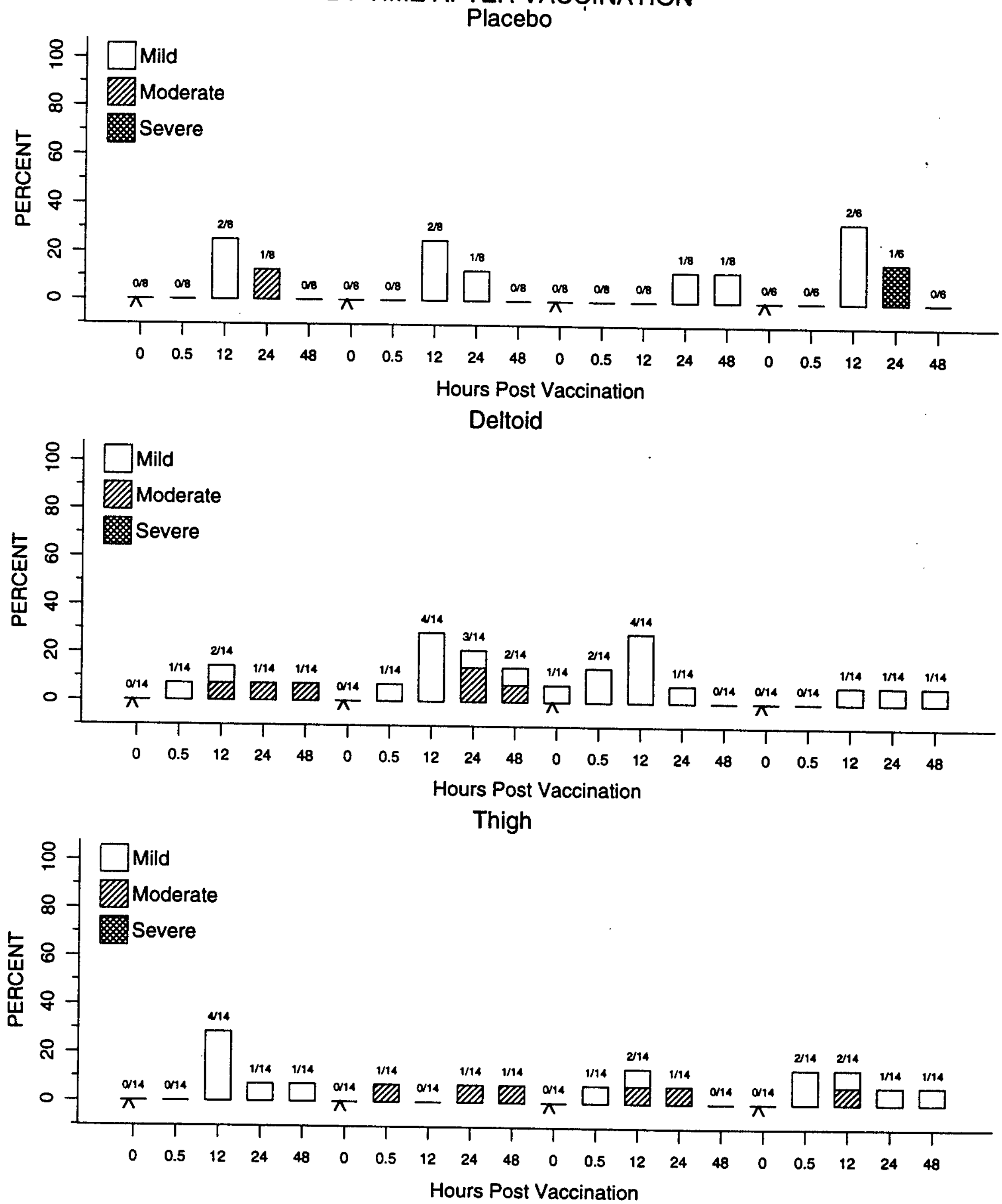


FIGURE 2

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]
MAXIUM PAIN AND TENDERNESS
BY TIME AFTER VACCINATION

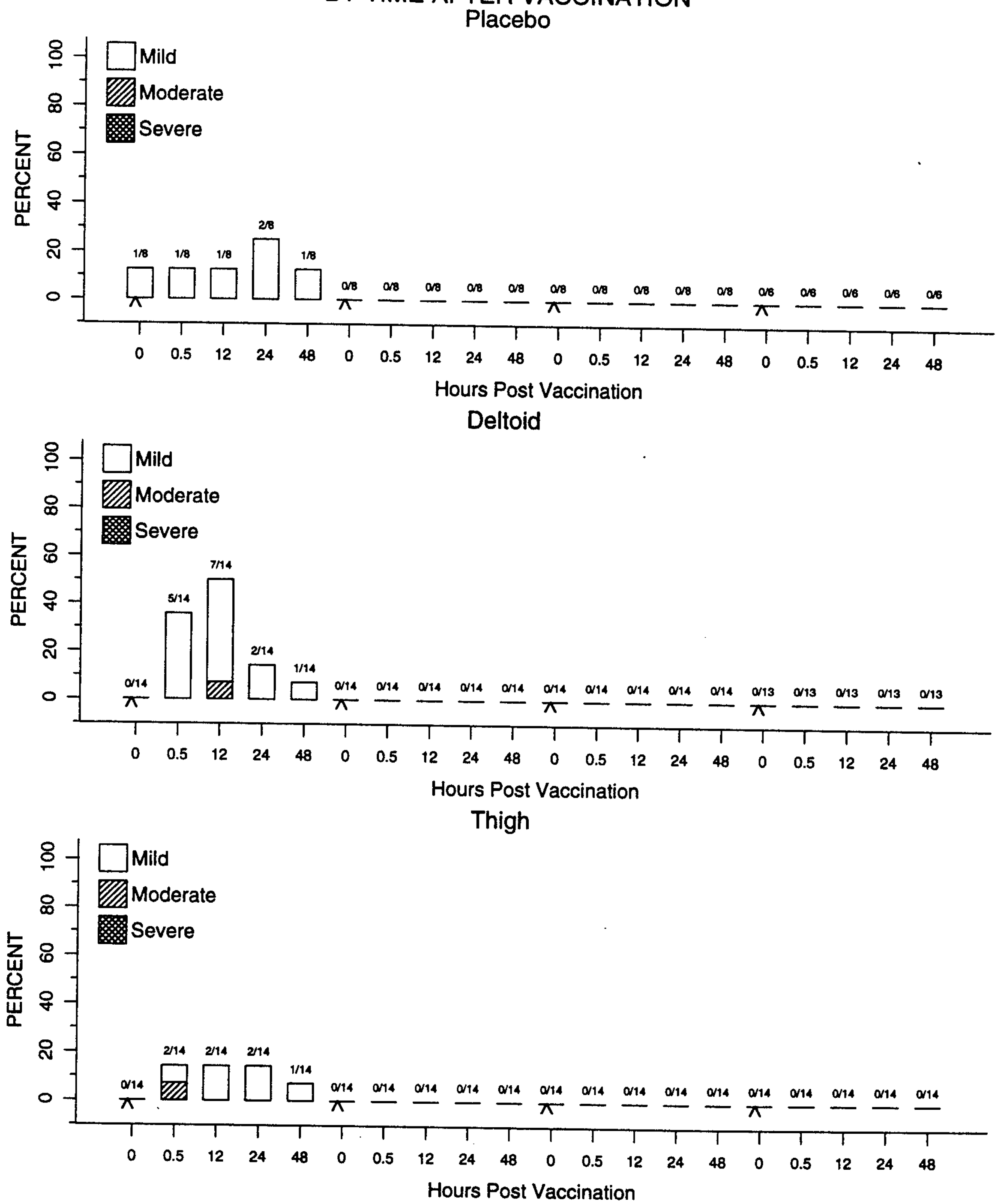


Figure 3

FIGURE 3

AIDS Vaccine Protocol 023 - HIV-1 MN PND Peptide and Microparticulate Immunogens [UBI]

ASC ELISPOT ASSAYS WITH TWO OR MORE ANTIGEN-SPECIFIC SPOTS

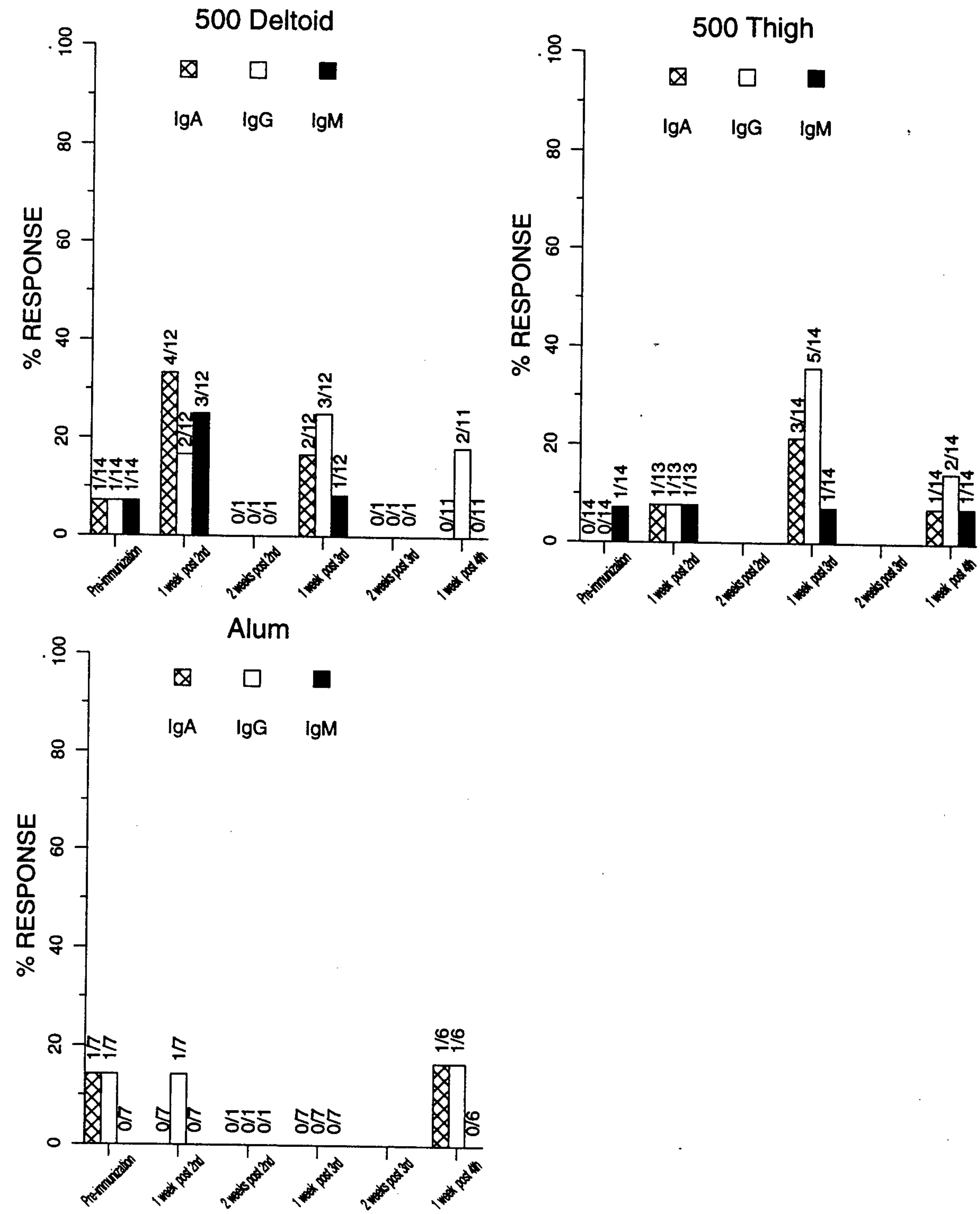


Figure 4

Figure 4: CTL of DNA Vaccine ± IL-12

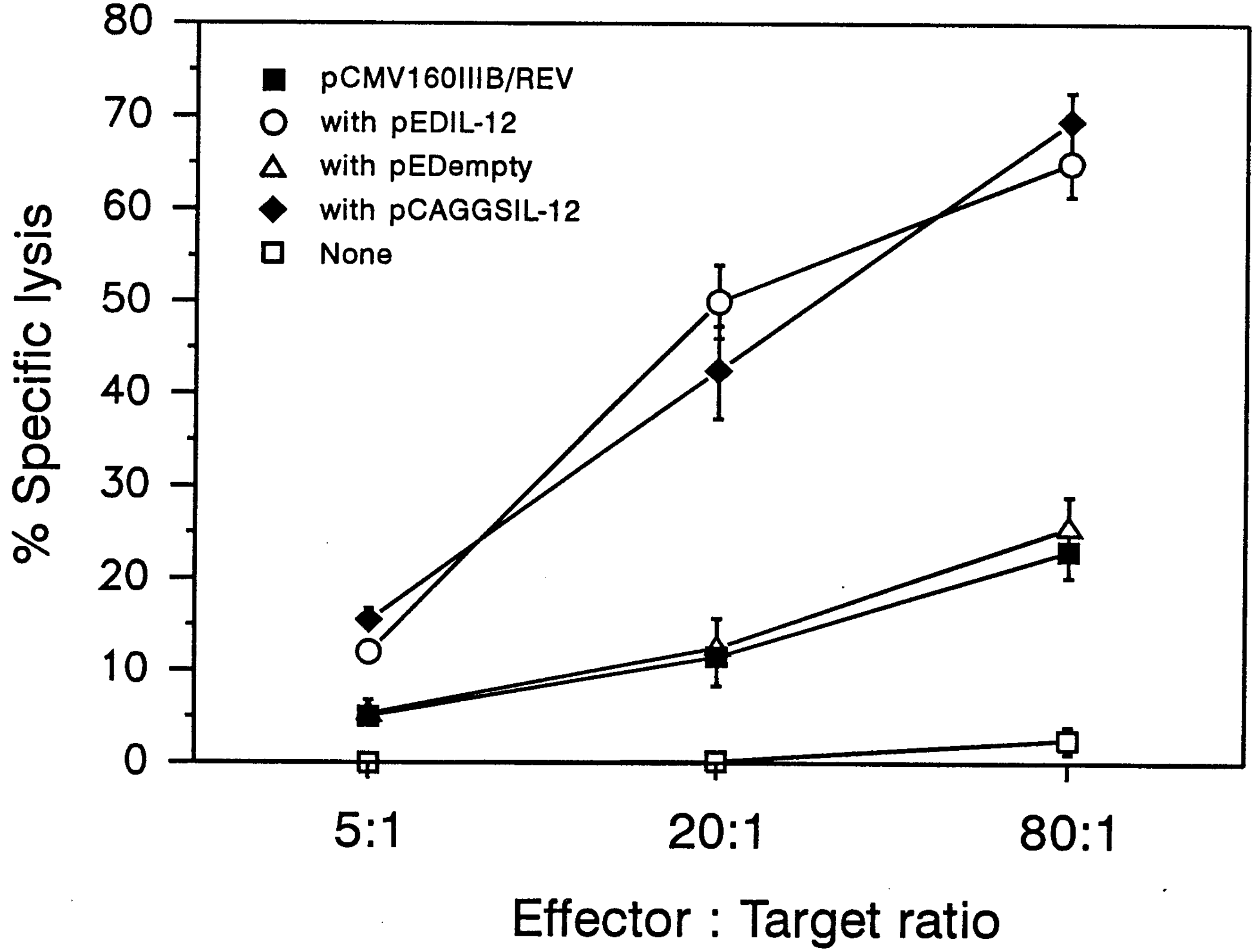
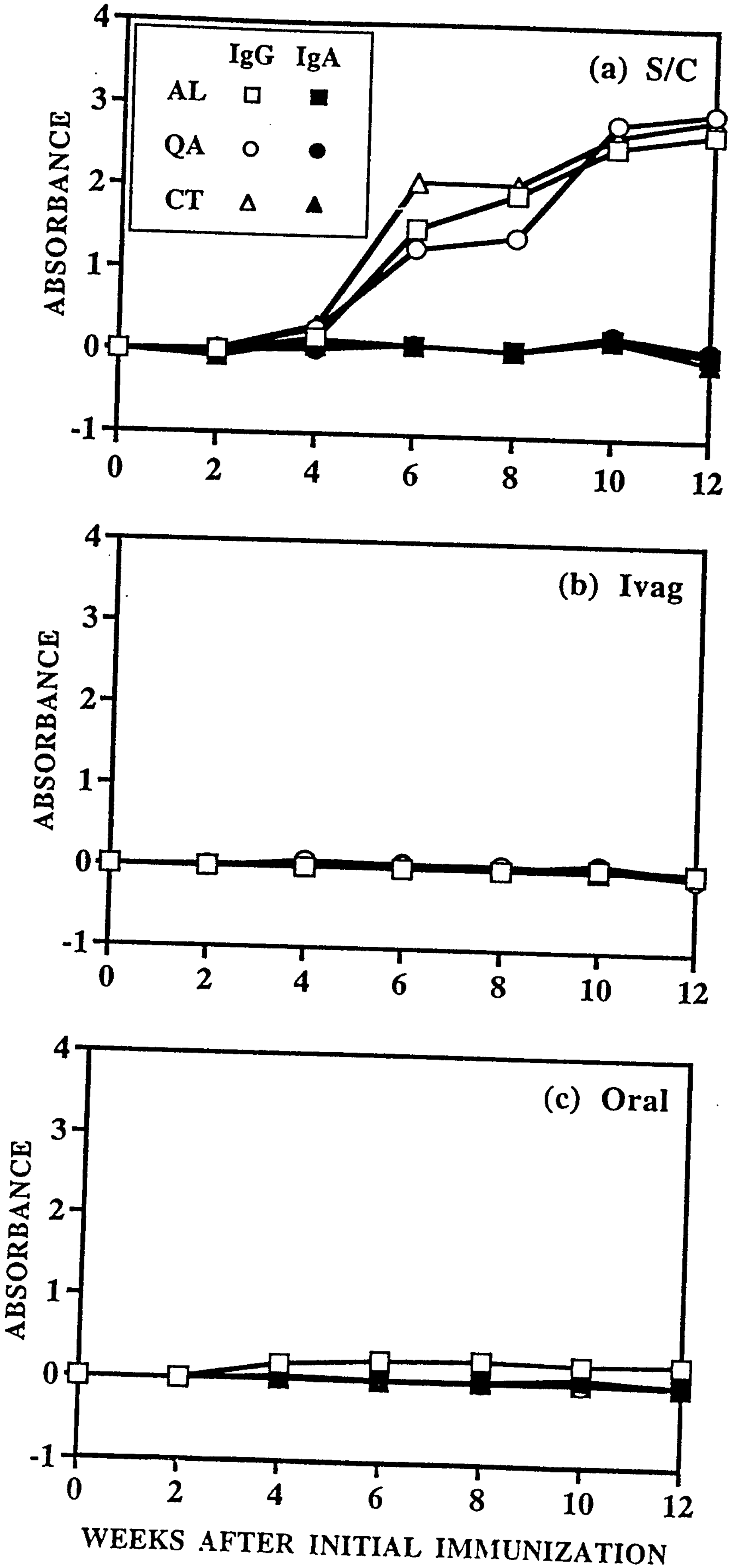
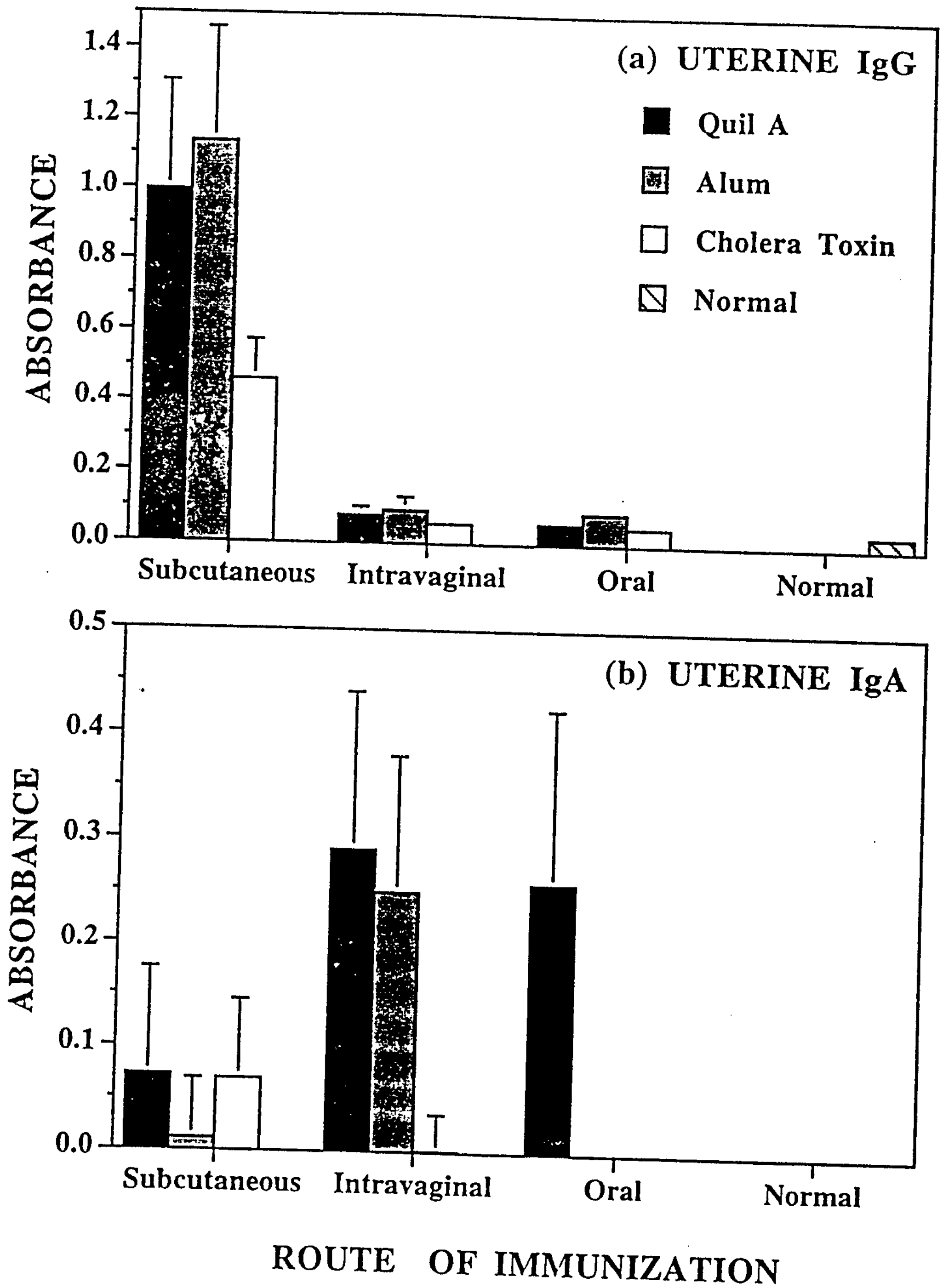


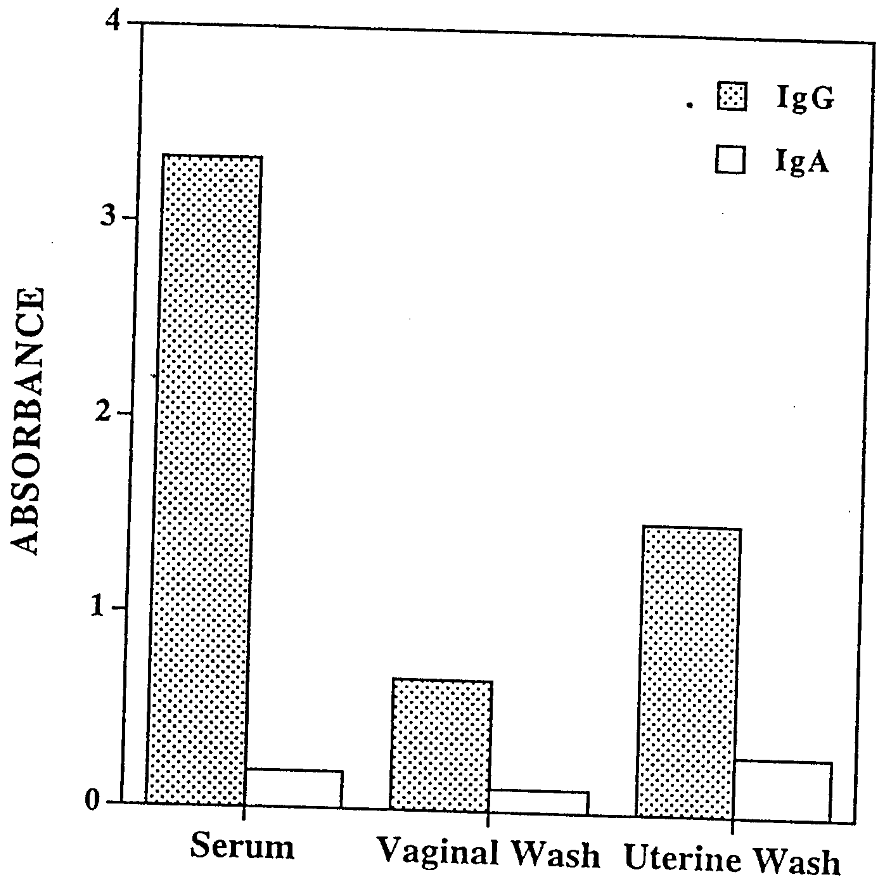
Figure 5



IgG IgA
AL
QA
CT
(a) S/C
(b) Ivag
(c) Oral
ABSORBANCE
4
3
2
1
0
-1
0
2
4
6
8
10
12
WEEKS AFTER INITIAL IMMUNIZATION

Figure 6


(a) UTERINE IgG
Quil A
Alum
Cholera Toxin
Normal
ABSORBANCE
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Subcutaneous
Intravaginal
Oral
Normal
(b) UTERINE IgA
ABSORBANCE
0.5
0.4
0.3
0.2
0.1
0.0
Subcutaneous
Intravaginal
Oral
Normal
ROUTE OF IMMUNIZATION


4
3
2
1
0
ABSORBANCE
IgG
IgA
Serum
Vaginal Wash
Uterine Wash